Retinal organoids on-a-chip: a micro-millifluidic bioreactor for long-term organoid maintenance.

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Funding Grants: Morphological and functional integration of stem cell derived retina organoid sheets into degenerating retina models

Public Summary:
This paper developed a micro-millifluidic bioreactor (with small chambers for each organoid) to maintain hESC-derived retinal organoids with continuous media supply. Organoids were analyzed by live imaging, single cell RNA sequencing, quantitative polymerase chain reaction , immunohistology, and electron microscopy. Bioreactor-cultured retinal organoids developed cell types and morphology comparable to static cultured ones and exhibited similar retinal genes expression levels. In summary, this bioreactor reduces shear stress on the organoids and significantly reduces labor for organoid maintenance.

Scientific Abstract:
Retinal degeneration is a leading cause of vision impairment and blindness worldwide and medical care for advanced disease does not exist. Stem cell-derived retinal organoids (RtOgs) became an emerging tool for tissue replacement therapy. However, existing RtOg production methods are highly heterogeneous. Controlled and predictable methodology and tools are needed to standardize RtOg production and maintenance. In this study, we designed a shear stress-free micro-millifluidic bioreactor for nearly labor-free retinal organoid maintenance. We used a stereolithography (SLA) 3D printer to fabricate a mold from which Polydimethylsiloxane (PDMS) was cast. We optimized the chip design using in silico simulations and in vitro evaluation to optimize mass transfer efficiency and concentration uniformity in each culture chamber. We successfully cultured RtOgs at three different differentiation stages (day 41, 88, and 128) on an optimized bioreactor chip for more than 1 month. We used different quantitative and qualitative techniques to fully characterize the RtOgs produced by static dish culture and bioreactor culture methods. By analyzing the results from phase contrast microscopy, single-cell RNA sequencing (scRNA seq), quantitative polymerase chain reaction (qPCR), immunohistology, and electron microscopy, we found that bioreactor-cultured RtOgs developed cell types and morphology comparable to static cultured ones and exhibited similar retinal genes expression levels. We also evaluated the metabolic activity of RtOgs in both groups using fluorescence lifetime imaging (FLIM), and found that the outer surface region of bioreactor cultured RtOgs had a comparable free/bound NADH ratio and overall lower long lifetime species (LLS) ratio than static cultured RtOgs during imaging. To summarize, we validated an automated micro-millifluidic device with significantly reduced shear stress to produce RtOgs of comparable quality to those maintained in conventional static culture.